

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 930 316 A1

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
21.07.1999 Bulletin 1999/29

(21) Application number: 98310267.4

(22) Date of filing: 15.12.1998

(51) Int. Cl.⁶: **C07K 16/02**, C07K 16/04,
C07K 16/10, C07K 16/12,
A61K 39/42, A61K 39/44
// (A61K39/44, 39:42, 35:74)

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: 19.01.1998 CZ 15898

(71) Applicant: **Medipharm CZ, s.r.o.**
693 01 Hustopece u Brna (CZ)

(72) Inventors:
• **Mican, Petr**
693 01 Hustopece u Brna (CZ)
• **Stepanek, Jan**
592 56 Zvole nad Perstynem (CZ)

(74) Representative:
Lawrence, John Gordon et al
McNeight & Lawrence
Regent House
Heaton Lane
Stockport, Cheshire SK4 1BS (GB)

(54) **Oral product for the prevention and treatment of infectious gastroenteritides in calves**

(57) Oral product for the prevention and therapy of infectious gastroenteritis in calves that contains antibodies to bovine rotavirus, bovine coronavirus and enterotoxigenic strains of *Escherichia coli* prepared from colostrum of immunized cows and/or egg yolks of immunized hens. It contains also a stabilized live culture of lactacidogenic bacteria. Method of production of antibodies to bovine rotavirus, bovine coronavirus and enterotoxigenic strains of *Escherichia coli* by immunization of cows and/or hens with antigens of bovine rotavirus, bovine coronavirus and enterotoxigenic strains of *Escherichia coli*, collection of colostrum from the immunized cows and/or egg yolks from the immunized hens and processing of these semi-products into the administration form, for instance by drying.

EP 0 930 316 A1

Description

[0001] The invention pertains to an oral product for the prevention and treatment of infectious gastroenteritis of calves.

[0002] Infections of the gastrointestinal tract rank among the most serious infectious diseases of young calves. Outbreaks are massive and affect particularly calves in the early postnatal period, i.e. at the age of ten to fourteen days.

[0003] The range of causative agents is rather wide, but major role is played by bovine rotavirus, bovine coronavirus and enterotoxigenic strains of *Escherichia coli* (referred to as ETEC henceforth). All these agents induce local infections limited to the mucosa of the small intestine. The infection route is oral and the source of the agents is the environment that is usually heavily contaminated.

[0004] Rotaviruses and coronaviruses penetrate into superficial cells of the intestinal mucosa and propagate there vigorously inducing degeneration and desquamation of enterocytes with the subsequent denudation and atrophy of the stroma of intestinal villi. ETEC adhere firmly to the surface of the intestinal mucosa and produce enterotoxin blocking entirely the function of the intestinal epithelial cells. The infection results in a loss of absorptive and metabolic potential of the intestinal mucosa, dysbacteriosis and hyperbiosis of the intestinal contents, transfer of tissue fluids into the intestinal lumen and general impairment of homeostasis. Clinical signs of the infection include moderate to severe diarrhoea, lethargy, dysorexia or anorexia, fever, dehydration and weight loss. Considerable economic losses result from growth retardation, high treatment costs and, last but not least, high mortality.

[0005] The approach to the protection against bacterial and viral infections of the gastrointestinal tract is determined by their local character. The most effective protection can be provided by specific antibodies to bovine rotavirus, bovine coronavirus and ETEC if present in time and in a sufficient concentration in the intestinal contents and on the surface of the small intestinal mucosa. The specific antibodies neutralize the potential of the agents to induce infection and prevent their adhesion onto the surface of the mucosa and penetration of rotaviruses and coronaviruses into epithelial cells of the intestinal mucosa.

[0006] Some strains of lactacidogenic bacteria (*Enterococcus* spp, *Lactobacillus* spp.) are known to prevent the adhesion of pathogenic bacteria to the surface of intestinal cells and to inhibit, on a competitive basis, their propagation in the intestinal contents. Calves reared under natural conditions receive the specific antibodies from colostrum that is also the source of bacteria providing a favourable microbial environment in the intestine. One of the incidental effects of implementation of advanced technologies in cattle husbandry are variations in the composition and concentration of colostral antibodies resulting in an impairment of natural protection of calves against gastrointestinal infections.

[0007] A wide range of preventive and therapeutic methods have been implemented to enhance the resistance of calves to diarrhoeic diseases and to reduce economic losses resulting therefrom.

[0008] Thus the formation of antibodies shed in colostrum is stimulated by immunization of dams with antigens of the three major causative agents.

[0009] However, antibodies present in blood at the time of immunization can partly or completely neutralize the administered antigens and thus to reduce or even eliminate the expected immunization effect. Moreover, relevant concentrations of specific antibodies persist in colostrum only during the first 24 to 48 hours after parturition and, consequently, the local passive immunity of the gastrointestinal tract is limited to the first three days of life.

[0010] Parenteral administration of specific antibodies to calves is almost ineffective, because the infection is limited to the small intestinal lumen, i.e. a place that is not accessible to parenterally administered antibodies.

[0011] Attempts were also done to protect calves by repeated oral administration of specific immunoglobulins prepared from colostrum of immunized cows.

[0012] However, the respective preparative methods are rather sophisticated and expensive and the efficacy of the specific immunoglobulins is insufficient in calves suffering from gastrointestinal infections manifested by diarrhoea of various severity and other symptoms. Therefore, this method has not found a wider application.

[0013] Also unsuccessful were the attempts to treat calves with antibodies prepared from blood serum of immunized animals. Orally administered serum immunoglobulins are rapidly degraded by gastric and intestinal enzymes.

[0014] Antibiotic therapy of infectious gastroenteritis in calves is rather difficult.

[0015] Antibiotics are ineffective against rotavirus and coronavirus infections and can only inhibit the propagation of ETEC in the intestine provided that the calves have been infected by a sensitive strain. Moreover, the administration of antibiotics results in an unwanted changes in the composition of the normal intestinal microbial flora, is often followed by a rapid development of resistance of the causative agent, and can suppress the activity of the immune system of the patients.

[0016] Favourable results have been reported in calves treated for infectious gastroenteritis with rehydration solutions. Although such solutions have no direct effect on the infection, they alleviate incidental signs resulting from dehydration and loss of electrolytes and reduce the mortality rate.

[0017] Probiotic products suppress the adherence and propagation of enteropathogenic and enterotoxigenic bacteria in the intestine, support the restoration of normal intestinal biocenosis and thus enhance the natural resistance of the patient to enteric infections. However, probiotics cannot influence intestinal infections induced by rotaviruses and coro-

naviruses.

[0018] The drawbacks of the conventional therapeutic methods can be eliminated to a considerable extent by treatment with the oral product for the prevention and treatment of infectious gastroenteritis as per invention, consisting in a combination of antibodies to bovine rotavirus, bovine coronavirus and ETEC prepared from colostrum of immunized cows and/or egg yolk of immunized hens with stabilized selected live cultures of lactacidogenic bacteria. Thus the specific passive immunotherapy is combined with the non-specific prophylactic effect of the probiotics. The active components of the product include a) specific antibodies to bovine rotavirus and coronavirus and ETEC that can neutralize these agents in the intestinal tract of calves by preventing their adhesion and blocking their penetration into intestinal cells and thus interrupting or alleviating the course of the infection, and b) stabilized live cultures of lactacidogenic bacteria *Enterococcus* spp and/or *Lactobacillus* spp. inhibiting, on competitive basis, the adherence to and propagation of enteropathogenic and enterotoxigenic bacteria in the intestinal tract, thus reducing the risk of bacterial sepsis, supporting the maintenance or restoration of microbial eubiosis also in calves affected by viral intestinal infections, and enhancing the local effect of specific antibodies. The basic components can be completed with further supportive components, such as vitamins, and excipients, the latter ensuring standard contents of the active components and homogeneity and long shelf life of the final product, and facilitate its administration.

[0019] The specific antibodies are prepared from colostrum of cows, or preferably from egg yolks of hens, immunized with antigens of bovine rotavirus (such as the CAMP strain V279), bovine coronavirus (such as the CAMP strain V362) and ETEC pathogenic for calves (such as CAMP strains 6231 through 6135).

[0020] The semi-products, in this case colostrum and liquid eggs, obtained from the immunized donors are homogenized and preserved by spray, fluid, or freeze drying. The latter technique should be preferred because it preserves fully the biological potential of the input materials. The resulting product is a white or yellowish loose powder. The minimal acceptable neutralization titre of antibodies to rotavirus and coronavirus in the dried colostrum or egg yolk is 16 for 100 TCID₅₀ (50% Tissue Culture Infectious Dose) per 0.05 g dried product.

[0021] The agglutination titre of antibodies to ETEC must reach at least 20/10⁸ inactivated bacterial cells per 0.05 g dried product.

[0022] The other active component of the product are stabilized live cultures of selected lactacidogenic strains of *Enterococcus* spp and/or *Lactobacillus* spp, such as *Enterococcus faecium* strain M74 - CCM 6226; *Lactobacillus casei* strain CCM 3775, or *Lactobacillus plantarum* strain CCM 3769. The probiotic component of the product is obtained by submersive pulsative culture of the respective strains. The bacterial mass resulting from the exponential growth phase is separated from the medium, supplemented with a cryoprotective agent and stabilized by freeze-drying. The concentration of live cells of lactacidogenic bacteria shall reach at least 50 x 10⁹ CFU (Colony Forming Units) per 1 g dry probiotic concentrate.

[0023] In order to increase the metabolic rate and enhance the resistance of the organism to infection, it is recommendable to supplement the product with vitamins A, D₃ and E concentrates in dry, oil or water-soluble form. The minimal daily doses should be 10 000 IU vitamin A, 1 000 IU vitamin D₃ and 15 mg vitamin E per animal.

[0024] The product is intended exclusively for oral administration to calves of various ages and therefore a paste form and a powder form have been devised.

[0025] The paste is intended above all for oral administration to newborn and suckled calves. The product is packaged in special plastic applicators allowing accurate and reliable dosage without any risk of aspiration.

[0026] The water-soluble powder form (pulis, premix) allows both individual dosage with a measuring jar and group dosage of the product mixed into skimmed milk, milk replacers or other milk-based combined feeds throughout the rearing period. The powder form is also suitable for mixing into rehydration solutions and other product intended for symptomatic therapy of infectious diarrhoea in calves.

[0027] The major excipient components of the paste are colloidal silicone hydrolysate and distilled monoglycerides that along with quality edible oil form a consistence with a molecular mesh ensuring a uniform dispersion of the active components. The personnel involved in paste processing must adhere to rules of aseptic work in all operations. Dried skimmed milk and dried whey combined with saccharose, glucose, sorbitol and lactose are used as excipients in the powder formula.

[0028] The major benefit of the oral product for the prevention and treatment of infectious gastroenteritis of calves is the fact that local immunity of the gastrointestinal tract against combined infections is induced by combined action of antibodies to rotaviruses, coronaviruses and ETEC and the inhibition effect of lactacidogenic bacteria against enteropathogenic and enterotoxigenic bacteria. Thus the product as per invention eliminates the drawbacks of the current preventive and therapeutic methods without affecting their efficacy. On the contrary, the induction of local immunity by the product enhances the vaccination effect and enhances the natural development of general immunity.

[0029] The product can be administered to any age group and particularly to newborn calves that are at the highest risk in the contaminated environment. Another advantage of the product is its composition, because it is produced from biological substances with nutritional characteristics identical with those of common components of calf feeds. The colostrum and egg yolk antibodies as active components of the product are resistant to low pH and proteolytic enzymes

and penetrate to the site of infection without harm. The paste, powder and premix forms allow optimal ways of administration to calves of any age categories.

Examples of materialization

[0030] The product for oral prevention and treatment of infectious gastroenteritides of calves is composed of the two basic active components, excipients and vitamin supplements processed into two administration forms, i.e. paste and powder.

[0031] The paste is intended for direct individual administration to calves, particularly newborn ones, while the water-soluble powder (pulvis or premix) is added to any type of calf feeds allowing both individual and group prevention and treatment.

[0032] Antibody donor animals (highly pregnant cows or hens) are immunized with antigens of bovine rotavirus (such as the CAPM strain V-279), bovine coronavirus (such as the CAPM strain V-352) and enterotoxigenic ETEC strains carrying the K 99 antigen (such as the CAPM strains 6231 through 6235) maintained in the Collection of Animal Pathogenic Microorganisms, Hudcova 70, 621 32 Brno, Czech Republic.

[0033] Selected bovine rotavirus and coronavirus strains are propagated in susceptible cell lines (such as MDBK), and the antigens are concentrated by centrifugation and/or ultracentrifugation. Individual ETEC strains are propagated in peptone, broth, yeast extract or another suitable growth medium.

[0034] At the end of the exponential growth phase, bacterial cells are separated from the medium and resuspended in physiological saline to obtain a standard concentration such as 10^4 per 1 ml. The viral and bacterial antigens are mixed, for example at a ratio of 20% rotavirus, 20% coronavirus and 60% ETEC, with an oil adjuvant and administered to pregnant cows in two doses before the expected parturition and/or repeatedly to lying hens at 21-day intervals for a period necessary for the production of specific antibodies.

[0035] The titres of the antibodies to rotavirus or coronavirus are checked using virus neutralization test and expressed in terms of reciprocal values of the dilution of colostrum or egg yolk suspension that neutralized 100 TCID₅₀ (50% Tissue Culture Infective Dose) of the respective virus. The titre of the antibodies to enterotoxigenic strains is expressed in terms of a reciprocal value of the colostrum and/or egg suspension dilution that, after mixing with the bacterial antigen, agglutinated 10^8 bacterial cells.

[0036] The materials collected from the immunized donor animals, i.e. colostrum and/or egg yolk suspension, are thoroughly homogenized and preserved by spray, fluid or freeze drying. The latter technique is preferred owing to the least effects on biological properties of the processed materials. The resulting yellowish powder is stored in a dry and cold room until further processing.

[0037] The most suitable material for the production of the concentrate of lactacidogenic bacteria are strains adhering well to the intestinal mucosa of calves and inhibiting markedly a wide range of enteropathogenic and enterotoxigenic microbes. Strains of *Enterococcus faecium* (such as M 74, CCM 6226), *Lactobacillus casei* (such as CCM 3775) or *Lactobacillus plantarum* (such as CCM 3769), maintained in the Czech Collection of Microorganisms (Tvrdého 14, 602 00, Brno, Czech Republic), are used in the product as per invention. The best method for obtaining the bacterial mass is submersive pulsative culture ensuring a high production of bacteria in the optimal log growth phase. The harvested bacterial cells are separated from the medium, for instance by centrifugation, and stabilized by freeze-drying. The dry bacterial mass with a minimum concentration of 50×10^9 cells of lactacidogenic bacteria per 1 g can be stored at -18 °C for prolonged periods. Before mixing with the other active components and excipients, this semi-product is warmed to room temperature. Data on packaging, administration and dosage of the product as per invention are given in the following examples:

Example 1

[0038] Paste is the most suitable and most effective form to be used in young calves, allowing easy and accurate dosing and avoiding the risk of aspiration of the product. Active components, including antibodies to rotavirus, coronavirus and ETEC, and the concentrate of lactacidogenic bacteria are mixed in the first step. A homogenous mixture of freeze-dried (dried) colostrum and/or freeze-dried (dried) egg yolks collected from the immunized donors, freeze-dried concentrates of cultures of *Enterococcus faecium* and/or *Lactobacillus casei*/*Lactobacillus plantarum* and vitamins A, D₃ and E is prepared. In the next step, this mixture is stepwise added into a homogenous mixture of excipients including quality edible oil, such as ground-nut oil, colloid silod hydrolysate, such as CABOSIL, and distilled monoglycerides, such as Myverol. All the active, supplement and excipient components are processed in a vacuum homogenizer and filled under aseptic conditions into plastic applicators with a volume of 40 or 80 ml. The mean oral daily dose of the paste is 1 to 5 ml per animal.

Table 1

Basic composition of the product in paste form	
Carrier components	w/w%
Quality edible oil (such as ground-nut oil)	40 - 60
Colloidal siloid (such as CABOSIL)	4 - 6
Distilled monoglycerides	1 - 3
Active components	
Dried colostrum or egg yolks collected from immunized animals	18 - 40
Probiotic concentrate $200 \cdot 10^9$ CFU per 1 g <i>Enterococcus faecium</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus plantarum</i>	4 - 5
Vitamin supplement	
Mixture of vitamin A, D ₃ and E concentrates	1 - 3

Example 2

[0039] The water-soluble powder form (pulvis, premix) is manufactured in standard blenders (such as Nautamix) with a system of horizontal and vertical mixing. In the first step, the active components including dried colostrum and/or dried egg yolks collected from immunized donor animals, and the freeze dried concentrate of lactacidogenic bacteria, are homogenized with the vitamin A, D₃ and E supplement in a smaller mixer. For the final processing, the mixture is transferred into the large blender containing a smaller amount of the excipient, and only after thorough mixing, the content is completed to the full volume and the mixing is finished. The product as per invention is packaged into 500-g alufan bags or plastic containers.

[0040] In the field, the dosage of the product as per invention is 5 to 15 g per calf and day, or 500 to 1 000 g per 1 ton dry milk replacer or milk-based combined feed.

Table 2

Basic composition of the product as per invention in the water-soluble powder (pulvis, premix) form	
Carrier components	w/w%
Dried instant milk or dried whey	19 - 35
Glucose, lactose, sorbitol, saccharose, starch	16 - 35
Active components	
Dried colostrum or egg yolks collected from immunized animals	20 - 60
Probiotic concentrate 50×10^9 CFU per 1 g <i>Enterococcus faecium</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus plantarum</i>	10 - 15
Vitamin supplement	
Mixture of vitamin A, D ₃ and E concentrates	

Claims

1. Oral product for the prevention and therapy of infectious gastroenteritis in calves characterized by the contents of antibodies to bovine rotavirus, bovine coronavirus and enterotoxigenic strains of *Escherichia coli* prepared from colostrum of immunized cows and/or egg yolks of immunized hens.
2. Oral product as per Claim 1, characterized by the content of a stabilized live culture of lactacidogenic bacteria.

3. Oral product as per Claim 2 characterized by a minimum titre of antibodies to rotavirus 64/100 TCID₅₀ and minimum agglutination titre of antibodies to enterotoxigenic strains of *Escherichia coli* 80 per 10⁵ inactivated bacterial cells per 1 g.
- 5 4. Product as per Claim 2, characterized by the fact that the live stabilized cultures of lactacidogenic bacteria are those of *Enterococcus* spp and/or *Lactobacillus* spp.
5. Product as per Claim 4 characterized by the concentration of the stabilized live cultures of lactacidogenic bacteria within the range of 5 x 10⁵ to 50 x 10⁹ viable bacterial cells per 1 g.
- 10 6. Product as per Claim 1, characterized by the contents of vitamins and excipients.
7. Product as per Claim 6 characterized by the minimum concentrations of vitamins A, D₃ and E 1 000 IU, 100 IU, and 3 mg, respectively per calf and day.
- 15 8. Product as per Claim 1 characterized by the content of 18 to 60 w/w% colostrum of immunized cows and/or egg yolks of immunized hens from a total
9. Product as per Claim 6 characterized by the following concentrations of the excipient components: edible oil 40 to 60 w/w%; silicone hydrolysate 4 to 6 w/w%; distilled monoglycerides 1 to 3 w/w% from a total amount of the product.
- 20 10. Product as per Claim 6 characterized by the contents of excipient components including glucose and/or saccharose and/or sorbitol in the final concentration of 16 to 35 w/w%, and dried milk and/or dried whey in the final concentration of 16 to 35 w/w% from a total amount of the product.
- 25 11. Method of production of antibodies as per Claims 1 through 10 characterized by immunization of cows and/or hens with antigens of bovine rotavirus, bovine coronavirus and enterotoxigenic strains of *Escherichia coli*, collection of colostrum from the immunized cows and/or egg yolks from the immunized hens and processing of these semi-products into the administration form, for instance by drying.
- 30 12. Method of antibody production as per Claim 11 characterized by the use of the strains CAMP V-279, CAMP V-352, and CAMP 6231 through 6235 as the antigen

ATTORNEY



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 98 31 0267

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	<p>ERHARD M H ET AL: "New possibilities in oral immunoprophylaxis of newborn diarrhea in calves—a field study using specific egg antibodies !. Neue Möglichkeiten in der oralen Immunprophylaxe der Neugeborenenstarrhoe des Kalbes—ein Feldversuch mit spezifischen Eiantikörpern."</p> <p>BERLINER UND MÜNCHENER TIERÄRZTLICHE WOCHENSCHRIFT, (1993 NOV) 106 (11) 383-7. JOURNAL CODE: 908. ISSN: 0005-9366., XP002102662</p> <p>GERMANY: Germany, Federal Republic of</p> <p>* page 383, left-hand column, line 1 - right-hand column, line 4 *</p> <p>* page 385, right-hand column, line 27-30 *</p> <p>* page 386, left-hand column, line 22 - right-hand column, line 2 *</p>	1-12	<p>C07K16/02</p> <p>C07K16/04</p> <p>C07K16/10</p> <p>C07K16/12</p> <p>A61K39/42</p> <p>A61K39/44</p> <p> //(A61K39/44, 39:42,35:74)</p>
X	<p>DATABASE BIOSIS</p> <p>BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US</p> <p>KRDZALIC P ET AL: "NEWLY DEVELOPED IMMUNOPROPHYLAXIS OF BEEF GASTROENTERITIC DISEASES USING POLYVALENT VIRAL-BACTERIAL VACCINE LACTOVAC."</p> <p>XP002102665</p> <p>* abstract *</p> <p>& VET GLAS, (1990) 44 (6), 449-454. CODEN: VEGLA1. ISSN: 0350-2457.,</p> <p style="text-align: center;">-/--</p>	1-12	<p>TECHNICAL FIELDS SEARCHED (Int.Cl.6)</p> <p>C07K</p> <p>A61K</p>
The present search report has been drawn up for all claims			
Place of search		Date of completion of the search	Examiner
THE HAGUE		12 May 1999	Covone, M
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X: particularly relevant if taken alone</p> <p>Y: particularly relevant if combined with another document of the same category</p> <p>A: technological background</p> <p>O: non-written disclosure</p> <p>P: intermediate document</p> <p>T: theory or principle underlying the invention</p> <p>E: earlier patent document, but published on, or after the filing date</p> <p>D: document cited in the application</p> <p>L: document cited for other reasons</p> <p>&: member of the same patent family, corresponding document</p>			

EPO FORM 1503 (3/82) (Pd/C01)



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 98 31 0267

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	WO 94 21284 A (PHARMA PACIFIC PTY LTD ;CHANDLER DAVID SPENCER (AU); REED BENJAMIN) 29 September 1994 * abstract * * page 2, line 8-25 * * page 6, line 13 - page 7, line 6 * * page 7, line 23-35 * * claims 12,16-18 * ---	1-12	
X	ERHARD M H ET AL.: "The lipopeptide, Pam(3)Cys-Ser-(Lys) (4): An alternative adjuvant to Freund's adjuvant for the immunisation of chicken to produce egg yolk antibodies" ATLA- ALTERNATIVES TO LABORATORY ANIMALS, vol. 25, no. 2, March 1997, pages 173-181, XP002102663 ENGLAND * abstract * * page 173, left-hand column, line 1-13 * * page 174, left-hand column, line 52 - right-hand column, line 10 * * page 178; table I * ---	1,3,8-12	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
X	REILLY R M ET AL.: "Oral delivery of antibodies - future pharmacokinetic trends" CLINICAL PHARMACOKINETICS, vol. 32, no. 4, April 1997, pages 313-323, XP002102664 * page 313, line 18 - page 314, line 5 * * page 320, right-hand column, line 29 - page 321, left-hand column, line 31 * * page 322, left-hand column, line 1-22 * -----	1,3,8-12	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 12 May 1999	Examiner Covone, M
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPO FORM 1503 (3.92) (P4/C21)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 98 31 0267

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

12-05-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9421284 A	29-09-1994	AU 673589 B	14-11-1996
		AU 6278794 A	11-10-1994
		EP 0706400 A	17-04-1996
		JP 8509965 T	22-10-1996
<hr/>			